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Bead Mill Homogenization and chemagic™ 360 Automated DNA Extraction Workflow for High-Definition PCR Detection of Tick-Borne Pathogens

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OMNI International, a PerkinElmer company

Bead Ruptor Elite™
Bead Mill Homogenizer

Summary

Second to mosquitos, ticks are considered the most dangerous arthropod vectors worldwide for the transmission of zoonotic infections [1]. Due to the blood meal processes in their life cycle, each tick is given numerous opportunities to pick up and spread new pathogens between their hosts. In addition to carrying numerous pathogens known to cause disease in humans and livestock, environmental factors are leading to expanding habitats of many tick species, resulting in previously unseen pathogens making their way into new environments via tick vectors [2].

This increasing burden of tick-borne disease has prompted numerous public health efforts for mitigation and epidemiologic surveillance of various disease-carrying ticks and the pathogens circulating within them. To fully evaluate the risk posed by a given tick population, their pathogen burden must be characterized and understood. The most sensitive and specific screening methods for pathogens seen in arthropod vectors currently rely on PCR-based testing; a tick may be screened by utilizing targeted primers for the presence of DNA from pathogens of interest. These screening measures can help medical and public health professionals identify, prepare for, and treat potential emerging infections in their communities.

These examination methods require DNA extraction from the tick, which can prove quite challenging due to their hard exoskeleton making complete lysis difficult to achieve. Conventional lysis methods typically involve manually bisecting the tick and allowing it to undergo enzymatic digestion for extended periods, ranging from a few hours to overnight incubations [3]. This extended digestion process prolongs the total time of the extraction for the DNA, delaying downstream analysis and significantly reducing throughput compared to other tissues processed similarly.

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Mechanical disruption can be used to achieve lysis in less time when compared with enzymatic lysis, and the yield of DNA can be dramatically increased. One of the most effective forms of mechanical lysis is bead-beating homogenization. Bead-beating has been shown to produce equivalent results upon repeated trials across a wide range of tissue and sample types [4].

In addition to complete sample lysis, the quality of DNA extracted also plays a significant role in the effectiveness of downstream applications. High yields and high-quality DNA are critical in providing accurate diagnostic and surveillance information on tick-borne pathogens. One way to increase accuracy and reproducibility is to introduce automation in as many steps as possible. The chemagic™ 360 instrument is an automated nucleic acid extractor; using its patented magnetic bead technology, the chemagic™ 360 instrument allows for highly reproducible sample processing for DNA extraction in less time and with greater accuracy than manual DNA extraction methods [4].

Detection of pathogens via traditional PCR or bioinformatic methods can be costly and time-consuming to verify each pathogen that may be in the sample. Using multiplexed assays to target multiple pathogens per reaction can dramatically reduce processing time and allow deeper coverage of a tick's actual pathogen burden. Herein, we utilize a multiplexed tick-borne pathogen (TBP) panel from ChromaCode to quantify the pathogen DNA of eight common tick pathogens relative to human diseases. ChromaCode leverages their proprietary High Definition PCR (HDPCR™) chemistry to detect nine of the most common tick borne pathogens in a single qPCR reaction.

The following study demonstrates a complete workflow incorporating the OMNI Bead Ruptor Elite™ Bead Mill Homogenizer, the chemagic™ 360 automated nucleic acid extractor and the HDPCR™ TBP Panel. Utilizing this workflow, we describe a method that can dramatically decrease the time required to screen a population of ticks while maintaining sensitivity and specificity of detection below clinically relevant copy numbers.

Materials and Methods

EQUIPMENT

- Bead Ruptor Elite™ Bead Mill Homogenizer (OMNI, Cat # 19-042E)
- 2 mL Hard Tissue Homogenizing Mix 2.8 mm Ceramic Beads (OMNI, Cat # 19-628)
- chemagic™ 360 Instrument (PerkinElmer, Cat # 2024-0020)
- chemagic™ DNA Stool 200 Kit H96 (PerkinElmer, Cat # CMG-1076)
- chemagic™ 96 Deep Well Plate (PerkinElmer, Cat # CMG-555-15)
- HDPCR™ Tick-Borne Pathogen (TBP) Panel For Research Use Only (PerkinElmer, Cat # SDX-62610)

PROCEDURE

96 pathogen-free *Amblyomma americanum* ticks were purchased from Oklahoma State University's Department of Entomology and Plant Pathology. These ticks were loaded individually into 2 mL Hard Tissue Homogenizing Tubes (OMNI, Cat # 19-628). Samples were then placed on the OMNI Bead Ruptor Elite™ Bead Mill Homogenizer (OMNI, Cat # 19-042E) and were homogenized at 6 m/s for two 60 second cycles with a 45 second dwell in between. The samples were centrifuged at 10,000 x g for 5 minutes after homogenization.

Once centrifugation was complete, the homogenate was processed using the chemagic™ DNA Stool Kit (Cat # CMG-1076) following the manufacturer's instructions. First, 800 µL of Lysis Buffer 1 was added to each tube, followed by 30 µL of Proteinase K and dilutions of the control pathogens of interest in triplicates. Dilutions were created based on commercially available stock at varying concentrations, and each control was serially diluted down to a concentration of only 1000, 500, and 50 copies/mL.

After adding buffers, each tube was quickly vortexed to ensure even distribution. Next, these vortexed tubes were incubated in a 70 °C water bath for 4 hours. Following incubation, the contents of the tubes were transferred into a 96-deep well plate (Cat # CMG-555-15). These plates were then loaded onto the chemagic™ 360 instrument, and the pre-programmed stool kit protocol was executed. The only deviation from the factory

protocol was the volume of the elution buffer, which was lowered to 50 µL as opposed to the recommended volume of 200 µL. After extraction, the plate contents were drawn into strip tubes and frozen at -20 °C until analysis with the HDPCR™ TBP Panel (PerkinElmer, Cat # SDX-62610).

RESULTS

Dry grinding of ticks resulted in a finely powdered homogenate suitable for liquid handling once buffers had been added. All tested pathogen were detected with copy numbers as low as 50 copies/mL, as shown in Table 1. Bartonella was used as an exclusivity control and was therefore not detected. A literature review was performed to examine the clinically relevant pathogen copy numbers found within wild ticks, as shown in Table 2. In these previous studies, detected pathogen copy numbers were generally much higher, ranging from a few hundred to several thousand copies/mL. Besides Bartonella, every pathogen screened for using the ChromaCode HDPCR TBP Panel was detected in at least two of the three replicates at each concentration at 50 copies/mL and showed 100 % detection for all pathogens at 500 copies/mL.

Reported Target	1000 cp Spike	500 cp Spike	50 cp Spike
Anaplasma phagocytophilum	3/3	3/3	3/3
Babesia microti	3/3	3/3	3/3
Borrelia Group 1	3/3	3/3	3/3
Ehrlichia chaffeensis	3/3	3/3	3/3
Borrelia Group 2	3/3	3/3	2/3
Borrelia miyamotoi	3/3	3/3	3/3
Rickettsia spp.	3/3	3/3	3/3
Ehrlichia ewingii	3/3	3/3	2/3
Ehrlichia muris eauclairensis	3/3	3/3	2/3
Negative Control	0/3	1/3	0/3

Table 1. Detection profile for nine tick-borne pathogens utilizing the ChromaCode HDPCR™ tick-borne pathogens kit on spiked samples. The spike columns represent how many replicates were detected at each dilution.

Pathogen	Copy Number per mL	Reference
Anaplasma phagocytophilum	339,000 – 609,000	5
Babesia microti	255,000 – 1,040,000	5
Borrelia Group 1	2,590 – 4,450	6
Bartonella Run Control	278 – 103,000	7
Ehrlichia chaffeensis	210 – 6,000	8
Borrelia Group 2	15.3 – 15.8	9
Borrelia miyamotoi	5,647 – 115,797	10
Rickettsia spp.	40– 10,000	11
Ehrlichia ewingii	None reported	
Ehrlichia muris eauclairensis	None reported	

Table 2. Pathogen copy number per milliliter for naturally seen pathogen burden as reported in the literature. No peer-reviewed reference was found accurately denoting the copy number of *Ehrlichia ewingii* or *Ehrlichia muris eauclairensis*. However, it can be estimated that a similar clinically relevant copy number to *Ehrlichia chaffeensis* based on the close similarities of the pathogen's life cycle and pathophysiology.

Conclusions

Herein, we have demonstrated the efficacy of our tick pathogen extraction workflow, incorporating bead-beating homogenization, automated DNA extraction, and HDPCR™ pathogen detection.

This workflow demonstrates optimized parameters for detecting sub-clinical levels of nine tick-borne human pathogens. In addition, this workflow significantly reduces extraction time compared with the conventional enzymatic digestion methods while providing high-quality purified DNA through automated extractions. The enzyme step alone is an overnight process, whereas the homogenizer requires only 3-4 hours of time, saving the end user 16-17 hours of experiment time.

Implementing this efficient and effective workflow will instead allow researchers, medical providers, and public health officials to better understand the pathogen burden in at-risk areas and more effectively implement prevention and treatment strategies.

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